

Grp 1643
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

John B. Harley and Judith A. James

Serial No: 08/781,296

Art Unit: 1643

Filed: January 13, 1997

Examiner: M. Zeman

For: **DIAGNOSTICS AND THERAPY OF EPSTEIN-BARR VIRUS IN
AUTOIMMUNE DISORDERS**

Assistant Commissioner for Patents
Washington, D.C. 20231

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**TRANSMITTAL OF EXECUTED DECLARATION
UNDER 37 C.F.R. § 1.132**

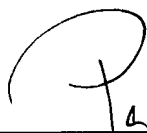
Sir:

In further response to the Office Action mailed June 24, 1998, applicants submit the executed Declaration Under 37 C.F.R. § 1.132 of John B. Harley. An unexecuted copy of the Declaration Under 37 C.F.R. § 1.132 was submitted along with the Amendment and Response to Office Action mailed December 24, 1998.

U.S.S.N.: 08/781,296
Filed: January 13, 1997
TRANSMITTAL OF SUBSTITUTE DECLARATION FOR
PATENT APPLICATION AND EXECUTED DECLARATION
UNDER 37 C.F.R. § 1.132

Allowance of claims 1-5 and 11-12, as amended, is earnestly solicited in view of the arguments made in the Amendment and Response to Office Action mailed June 24, 1998, and the attached Declaration.

Respectfully submitted,



Patrea L. Pabst
Reg. No. 31,284

Date: January 6, 1999
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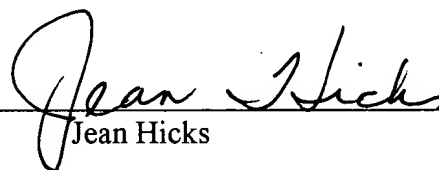
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CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with the United States Postal Service on the date shown below with sufficient postage as first-class mail in an envelope addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231.



Jean Hicks

Date: January 6, 1999



Part of
#20 1/2

Applicants: John B. Harley and Judith A. James

Serial No: 08/781,296

Examiner: M. Zeman

Filing date: January 13, 1997

Art Unit: 1643

For: "*Diagnostics and Therapy of Epstein-Barr Virus in Autoimmune Disorders*"

Assistant Commissioner of Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. §1.132 OF DR. JOHN HARLEY

Sir:

I, John B. Harley, hereby declare that:

1. I am an inventor of the subject matter described and claimed in the above-identified patent application.
2. I received my B.S. in Physics and Chemistry from Dickinson College, Carlisle, Pennsylvania in 1971. I received my Ph.D. in Biochemistry from the University of Pennsylvania, Philadelphia, Pennsylvania, in 1976. I received my M.D. from the University of Pennsylvania, Philadelphia, Pennsylvania in 1974. I am Board Certified as a Diplomate of the American Board of Internal Medicine, American Board of Rheumatology and the American Board of Allergy and Immunology. I am currently the James R. McEldowney Professor of Immunology and Professor of Medicine, Adjunct Professor Department of Microbiology and Adjunct Professor Department of Pathology at the University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma; and A Member, Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma. My Curriculum Vitae is attached.

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Studies Indicating Rheumatoid Arthritis is Characterized by B Cell Spreading

3. We have conducted additional studies which show an association between Epstein-Barr virus and another autoimmune disorder, inflammatory polyarthritis.

To preliminarily address the issue of association we obtained sera from some of the cases and controls in the Norfolk Arthritis Register. This is an RA disease inception study where all of the available patients in this region of England with inflammatory polyarthritis were enrolled as close to presentation as was possible along with appropriate controls. The overwhelming majority of these cases satisfy the 1987 Classification Criteria for RA. Our collaborator in this effort, Professor Alan Silman, has sent us 174 blinded sera from this resource collected at enrollment interview of patients with polyarthritis, thought to be typical for the presentation of RA, and controls. We assayed these blinded serum specimens for anti-EBV VCA IgG (Epstein-Barr virus Viral Capsid Antigen), anti-CMV IgG (cytomegalovirus), anti-HSV-1 IgG (herpes simplex virus, type 1), and anti-HSV-2 IgG (herpes simplex virus, type 2), using the methods described in our patent application.

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Table 1. Association of anti-EBV VCA IgG with inflammatory polyarthritis with sera from the Norfolk Arthritis Register.

	<u>Anti-EBV-VCA IgG</u>		<u>Anti-CMV IgG</u>		<u>Anti-HSV-1 IgG</u>		<u>Anti-HSV-2 IgG</u>	
	+	-	+	-	+	-	+	-
RA	78	2	30	50	41	39	26	65
Controls	78	13	40	51	46	45	23	57
Odds Ratio	6.5		0.8		1.0		1.0	
χ^2	7.4		0.7		0.0		0.0	
P value	<0.01		NS		NS		NS	

NS=not significant. Analysis is done by the McNemar test.

Upon receipt of the anti-viral assay results in Manchester the code was broken and the analysis is proceeding in both centers. The results show support for association of EBV seropositivity just after presentation with inflammatory polyarthritis.

There is no obvious association of inflammatory arthritis with seropositivity against HSV-1, HSV-2 or CMV. There is however, a strong but not perfect association of anti-EBV IgG with the cases. While significant, these numbers are small and warrant being established in a group of sufficient size to more accurately estimate the odds ratio and to explore the RA patients who are seronegative with regard to EBV.

One of the possible artifacts is that something about RA generates false positives in

the anti-EBV VCA IgG assay. The obvious candidate for such nefarious interference is rheumatoid factor (RF). Data in the literature support there being no interference between the anti-EBV VCA IgG and RF. Nevertheless, we have explored this issue by removing approximately 90% of the RF activity by solid phase absorption with human IgG from four RA patients who were RF seropositive and from two RF negative control sera. The anti-EBV VCA IgG decreased by averages of 21% in the RF positive RA sera and 27% in the RF negative control sera. In no RA case nor control did the serologic status of anti-EBV-VCA IgG change from positive to negative (or vice versa). The simultaneous reduction in the antibody titer against a control antigen (varicella zoster) was exactly the same, 21% and 27%, respectively. By our data and the experience in the literature we are led to the conclusion that RF does not interfere with the anti-EBV-VCA IgG assay.

4. We also have preliminary data in RA with EBNA-1. We have constructed the 443 unique overlapping octapeptides from EBNA-1 used to analyze the specificity of response in sera from patients with lupus, as described in our patent applications, but have used them to evaluate the fine specificity of the anti-EBNA-1 IgG antibody response in an RA patient and a normal control (Figure 1). There are 641 amino acids in the sequence, but some are repetitive and therefore were made only once. This approach has been applied in a number of other studies from our laboratory.

Figure 1 shows the binding of a rheumatoid factor positive RA patient serum (A) and a sex, race and age matched negative control (B) to the 443 unique overlapping octapeptides of EBNA-1. The RA patient mounts a very diversified response against EBNA-1. The normal

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control, on the other hand, has a much more limited response. Note the apparently identical responses binding peptides near peptide number 210 and 260 in both the normal and the RA patient. Meanwhile, the RA patient has anti-EBNA-1 antibodies against a number of peptides not bound by the normal. Among these are octapeptides numbered 244 to 347 (↓) which contain RLPFG which is crossreactive with collagen.

Many more of the EBNA-1 derived peptides are bound by the RA patient than are bound by the control. At the same time the RA patient binds all of those bound by the control. These results are, perhaps, consistent with the much higher frequency of precipitins against EBNA-1 in RA found in the precipitin studies in the literature relative to those results using an immunofluorescence assay.

These data present the opportunity to define aspects of the fine specificity of the RA anti-EBV B cell response which are qualitatively unique to RA. Note that the RA patient binds all of the peptides bound by the matched control. In addition, there are a number of regions (octapeptides numbered 25 to 75, and those near 175, 275, 320, 345, 360, 385, 390, 410 and 420) which are bound by the RA patient serum and not by the normal control. After collecting data from a number of such pairs and other inflammatory disease controls we could identify those relatively specific for RA. Interestingly, Octapeptides numbered 344 to 347 contain RLPFG which has shown to be immunologically crossreactive with collagen by Cook, et al. *J. Autoimmun.* 11:205-211 (1998) and is, therefore, a strong candidate for being a relatively RA specific anti-EBV response

5. The literature and our data as presented above lead us to suspect that the following model

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or scenario concerning RA is true. Because of genetic factors (e.g. HLA-DR4) some individuals have increased risk of becoming affected with RA. Some RA patients have an abortive infection of their synovium with EBV. These elements then are responsible for or predictive of the following: an association of EBV with RA (Table 2), humoral immune abnormalities in RA directed against EBV (Table 1 and Figure 1), the relative absence of specific anti-EBV T cell activity in the peripheral blood (failure to suppress transformed B cell outgrowth) of RA patients, and the concentration of anti-EBV early antigen T cell activity in RA synovium of some patients.

Additional Studies Demonstrating Induction of Autoimmunity in Animal Models

6. The Examiner has requested data showing that one can prevent or alleviate symptoms of autoimmune disorders using our claimed compositions. These data in human are not currently available. However, studies have been conducted, as described in our application that show that certain peptide sequences present in EBV peptides can induce autoimmune disease. We have also conducted studies showing that we can induce tolerance to these same peptides, so that autoimmune disease is more difficult to induce.

7. Two different immunization schedules were used as described in Exhibit 3. One, Schedule A, in which rabbits were injected on days 0, 26, 53, 99 and 152, succeeded in inducing anti-Sm autoantibodies every time it was employed. The second schedule, Schedule B, in which animals were injected on days 0, 8, 22, 67, 151, 275, and 317, failed to induce anti-Sm autoimmunity in two rabbits and resulted in a much slower and less vigorous anti-Sm response in the remaining animals. These results were published by Scofield, et al. *J. Invest. Med.* 45:200A (1997). We believe the critical difference is with the first few injections. Nevertheless, Schedule

B resulted in the development of B cell epitope spreading to other regions of Sm B/B' at a much slower rate than animals immunized with Schedule A and none of these Schedule B animals showed evidence of becoming ill with a lupus-like illness. Indeed, two of the six Schedule B animals developed no detectable anti-Sm B/B' autoantibodies, indicating that they were tolerized to Sm B/B'. In fact, all of the Schedule A animals in these experiments and all of the rabbits immunized since then with a schedule similar to Schedule A using PPPGMRPP-Map developed anti-spliceosomal autoimmunity manifested by the presence of anti-Sm B/B' autoantibodies. We had used Schedule B to accelerate the development of autoantibodies and instead this strategy did the opposite. It is known to one skilled in the art that boosting early has the potential to induce tolerance.

8. A schedule similar to Schedule B was used for baboon immunization, called Schedule B* (Table 1). Again, only one of the two animals developed anti-Sm B/B' autoantibodies. Neither of these animals developed clinical illness, also consistent with the possibility that their immune systems were responding in ways similar to the rabbits immunized (and presumably tolerized) with Schedule B. These results were published by Arbuckle, et al., *J. Exp. Med.* 46, 18-22 (1997).

9. We have shown that genetic constitution appears to be very important, based on work done in mice. Using a schedule similar to Schedule A, above, we immunized 13 different mouse strains with PPPGMRPP. All strains made some antibody against the peptide of immunization, but only six showed B cell epitope spreading and evidence for the development of anti-spliceosomal autoimmunity. Subsequent experiments have suggested that the difference

between the AKR/J (responder) and the C57BL/J strains (non-responders) operates in an autosomal dominant fashion and is due to a difference on mouse chromosome 4. These results and analysis are reported by James and Harley in *J. Immunol.* 160, 502-508 (1998) and "Preliminary genetic linkage of lupus autoimmune spreading with a polymorphic lymphocyte cell-surface antigen" *Arthritis Rheum.* 39, S216 (1996). In aggregate, these data show that the genetic constitution influences the capability for the development of anti-spliceosomal autoimmunity.

10. The sequence PSQQVMTP-Map is taken from a region of Sm B/B' which is not normally antigenic. Unlike PPPGMRPP-Map, this second sequence does not induce anti-spliceosomal autoimmunity. This outcome suggests that the result varies according to the structure of the immunogen and is consistent with the possibility that other sequences, such as PSQQVMTP would be capable of interfering with or otherwise preventing anti-spliceosomal autoimmunity.

11. A dose response experiment in the AJ and AKR/J strains shows that the optimal responses for inducing anti-spliceosomal autoimmunity is close to the 100 mcg dose of PPPGMRPP-Map that had previously been used. At the lowest and highest dose neither strains developed spliceosomal autoimmunity while both did at the 100 mcg dose of PPPGNRPP-Map. This is consistent with high and low zone tolerance operating in this system.

12. In another study, we retained the protective group on the arginine in the PPPGMRPP-Map. When one rabbit or five A/J mice were immunized with these peptides, no anti-spliceosomal autoantibody was detected. Anti-spliceosomal autoimmunity in the form of anti-

spliceosomal autoantibodies were observed when these peptide preparations were mixed with PPGMRPP-Map prepared in the ordinary way, in which the protective group was removed.

13. We defined two peptides in one of the binding patterns in the nRNP A protein called A3 and A6. Immunization of rabbits or mice with A3 leads to a limited response without evidence for spliceosomal autoimmunity. Rabbit or mouse immunization with A6 leads to B cell epitope spreading and spliceosomal autoimmunity. This is another example of the specificity of the response and suggests that manipulation of the peptide composition will allow control of the immune response.

14. Additional rabbits were immunized on a third schedule, Schedule C, in which they were immunized only once. In this case, the immune response was restricted to the peptide only and extinguishes over time. No spliceosomal autoimmunity develops. This result is consistent with a partial immunization being used to induce tolerance and thereby prevent the lupus-like autoimmunity which would otherwise result.

15. In summary, the results described in paragraphs 7-14, indicate that this model of lupus autoimmunity is dependent upon the immunization schedule and the structure of the peptide in ways which are consistent with the possibility that the anti-spliceosomal autoimmune response and the lupus-like illness it is associated with it could be blocked through the induction of tolerance.

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DECLARATION OF DR. JOHN B. HARLEY

16. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further, that these statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date:

Jan. 4, 1999

John B. Harley
John B. Harley



Curriculum Vitae
John B. Harley, M.D., Ph.D.

Page 2

- 1979 - 1982 Clinical Associate, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland
- 1977 - 1979 Intern and Resident in Internal Medicine, Yale University, New Haven, Connecticut
- 1976 - 1977 Postdoctoral Fellow, Department of Tumor Immunology, Imperial Cancer Research Fund Laboratories, London, England
- 1975 - 1977 Postdoctoral Fellowship from the National Institute of Allergy and Infectious Diseases with Departments of Microbiology, Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania
- 1974 Assistantship in Physiology, University of Pennsylvania
Philadelphia, Pennsylvania
- 1970 Co-Director, Halfway House, Dauphin County Unit, Harrisburg State Hospital, Harrisburg, Pennsylvania

Military Service

- July 1, 1979 - Active Duty, Public Health Service:
June 30, 1982 Senior Assistant Surgeon (03), July 1, 1979;
Surgeon (04), February 1, 1980

Board Certification

- 1979 Diplomate of the American Board of Internal Medicine
1982 Diplomate of the American Board of Rheumatology
1983 Diplomate of the American Board of Allergy and Immunology

Scientific Recognition

- Landis-Mohler Prize in Physics (1968)
A. J. Carnell Fellowship, Medicine (1972)
Edgar J. Kaufman Fellowship, Biochemistry (1973)
Balduin-Lucke Award in Medical Research (1974)
Arthritis Investigator Award, Arthritis Foundation (1985)
Young Investigator Award, National Institutes of Health (1985)
Arthritis Foundation Travel Award (1986)
Appointed by Governor George Nigh to the Oklahoma Health Research Committee (1986)
Alumni Research Scholar, University of Oklahoma College of Medicine (1987)
Clinical Investigator Award, Veterans Administration (1987)
Recognition of Outstanding Contribution, Faculty Senate, University of Oklahoma Health Sciences Center (1988)
Provost Research Award, University of Oklahoma Health Sciences Center (1988)
ASHI Scholar Award, American Society Histocompatibility and Immunogenetics (1989)

CURRICULUM VITAE

John B. Harley, M.D., Ph.D.

Personal Data

Date of Birth: September 13, 1949

Married: Barbara West on August 8, 1972

Children: Andrew West Harley, September 5, 1979
Isaac Thomas West Harley, January 2, 1982Education

1976	Ph.D.	University of Pennsylvania, Philadelphia, Pennsylvania (Biochemistry)
1974	M.D.	University of Pennsylvania, Philadelphia, Pennsylvania
1971	B.S.	Dickinson College, Carlisle, Pennsylvania (Physics and Chemistry)

Professional Experience

1982 - Present	James R. McEldowney Professor of Immunology and Professor of Medicine (1992 to present), Associate Professor (1986 to 1992), Assistant Professor (1982 to 1986), Department of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
1982 - Present	Member (1998 to present) Associate Member (1989 to present), Affiliated Associate Member (1986 to 1989), Affiliated Assistant Member (1982 to 1986), Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma
1983 - Present	Adjunct Professor (1992 to present), Adjunct Associate Professor (1988 to 1992), Adjunct Assistant Professor (1983 to 1988), Department of Microbiology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
1996 - Present	Adjunct Professor, Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma
1982 - Present	Staff Physician (1982, 1984 to 1987 and 1992 to present), Clinical Investigator (1987 to 1992), Immunology Section, Medical Service, Veterans Administration Medical Center, Oklahoma City, Oklahoma
1981 - 1982	Postdoctoral Fellow in Rheumatology, Arthritis Branch, National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland

John B. Harley, M.D., Ph.D.

Prix de la Meilleure Communication Scientifique, Premieres Journees Bretonnes D'Autoimmune (1990, shared with L. Jacobsson, B. Hansen, K. Hardgrave and R. Manthorpe)
Howard and Martha Holley Research Prize, American College of Rheumatology and the University of Alabama in Birmingham (1991)
James R. McEldowney Professorship in Immunology, University of Oklahoma (1992)
Wallace Graham Lecturer, University of Ottawa (1992)
Philip Hench Award, Association of Military Surgeons of the United States (1996)

Professional Societies

American Federation for Medical Research (1980)
American Rheumatism Association (1983)
Oklahoma Academy of Science (1983)
Oklahoma Rheumatism Society (1983)
American Association of Immunologists (1984)
American Society for Histocompatibility and Immunogenetics (1987)
Central Society for Clinical Investigation (1987)
Oklahoma Allergy Society (1988)
American Society for Clinical Investigation (1990)
American Association for the Advancement of Science (1991)
American Society for Human Genetics (1993)
Clinical Immunology Society (1995)

Institutional Duties

Fleming Scholar Committee, Oklahoma Medical Research Foundation, Chairman (1983-1986), Member (1983-present)
Electron Paramagnetic Resonance Facility Committee, Oklahoma Medical Research Foundation, Member (1985-1989)
Pharmacy and Therapeutics Committee, Oklahoma Memorial Hospital, Member (1983-1991); Chairman (1984-1989)
University Council on Faculty Awards and Honors, University of Oklahoma (1988-1991)
Oklahoma Medical Research Foundation Lecture Series Committee, Member (1989-1991)
MD/PhD Program, College of Medicine, University of Oklahoma, Director (1989-present), MD/PhD Advisory Committee (1989-present)
Department of Medicine Executive Committee, Member representing Associate Professors (1989-1991) and Professors (1993-1995)
Honorary Degree Screening Committee, University of Oklahoma, Member (1990-1993)
Center for Molecular Medicine, University of Oklahoma, Member (1991-)
Search Committee, Associate Chief of Staff for Research, Department of Veterans Affairs Medical Center, Member (1991-1992).
Search Committee for Gammill Chair in Polycystic Kidney Disease, Member (1996-1997)

Professional Service

American Federation of Clinical Research, Senator representing the University of Oklahoma (1983-1986)
Scientific Advisory Committee, Oklahoma Lupus Association, Member (1986-)

Rheumatology Abstract Review Committee, American Federation for Clinical Research, Member (1986)
National Subcommittee for Chapter Grant Review, Arthritis Foundation, Member (1985-1987).
Lupus Council, Arthritis Foundation, Member (1986-1990)
Oklahoma Health Research Committee, Member (1986-1995, Appointee of Governor George Nigh); Chairman (1986-1995)
Humoral Immunology Abstract Review Committee, American Rheumatism Association, Member (1987-1988)
Ad hoc Site-Visit Review Group, National Institute of Arthritis and Musculoskeletal and Skin Disease, Member (1987)
N.A.T.O. Collaborative Research Program, Collaborators on Complete Congenital Heart Block, Coordinator (1986-1988)
Scientific Review Committee, Presbyterian Health Foundation, Approved But Not Funded Program, Member (1988-1989)
Centers of Excellence Planning Committee, OCAST, Substitute Member (1988)
Two to Grown On, Executive Committee, Member (1988, Appointee of Governor Henry Bellmon)
By-Laws Committee for the Professional Practice Plan, Chairman (1988)
Systemic Lupus Erythematosus Etiology and Pathogenesis, Abstract Review Committee, American College of Rheumatology, Member (1989-1992)
Autoimmunity Minisymposium, Federation of the American Societies of Experimental Biology, American Association of Immunologists, Chairman (1990)
Editorial Board, Arthritis and Rheumatism (1990-1993)
Arthritis Research Council Special Site Visit Review Committee, National Institute for Arthritis and Musculoskeletal Diseases, Member (1990)
AFCR, ASCI, AAP National Meeting, Abstract Selection Committee, Chairman for Rheumatology (1991)
Editorial Board, Clinical and Experimental Rheumatology (1991-1994)
Program Specialist, Merit Review Board in Immunology, Research Services, U.S. Department of Veterans Affairs (1991-1994 and 1994-1997)
Reviewer's Reserve, National Institutes of Health, member (1992-1993)
General Medicine A Study Section, Division of Research Grants, National Institute of Health, ad hoc member (1992)
Specialized Centers of Research in Rheumatoid Arthritis Study Section, National Institute for Arthritis, Musculoskeletal and Skin Disease, Member (1992)
Experimental Immunology Study Section for Request for Applications "The effects of silicone on the immune response and in autoimmunity", Division of Research Grants, National Institutes of Health, Member (1993)
General Medicine A-2 Ad Hoc Review Section (AHR-M2), National Institutes of Health, Member (1993)
General Medicine A Study Section, Division of Research Grants, National Institutes of Health, Member (1993-1997)
Internal Administrative Advisor to "Sensory Integration Relevant to Cardiopulmonary Function" a Program Project proposal, Robert D. Foreman, Principal Investigator (1994)
Lupus Foundation of America, Medical Council, Member (1994-1996)
Clinical Sciences Special Emphasis Panel, National Institutes of Health, Member (1994)
Special Review Committee, National Institutes of Arthritis, Musculoskeletal and Skin Disease, Chairman (1995)

John B. Harley, M.D., Ph.D.

Oklahoma Center for the Advancement of Science and Technology (OCAST), Member of the Board of Directors (1995-present; Appointee of Governor Frank Keating); Vice Chairman (1995-1996 and 1996-1997)

American Association of Immunologists, Member of the Public Affairs Committee (1996-Present)
Ad hoc Technical Evaluation Group for RFP NIH-NIAMS-96-001, "Gene Therapy for Rheumatic and Skin Diseases", National Institute of Arthritis, Musculoskeletal and Skin Diseases, Chairman (1996)

University of Michigan Lupus SCOR, Member, Scientific and Administrative Advisory Committee (1997 - present)

NIAID Task Force on Immunology, Member (1997-1998)

Clinical Infrastructure Task Force, S.L.E. Foundation (1998-present)

Attending Physician

Oklahoma Memorial Hospital, Oklahoma City

U.S. Department of Veterans Affairs Medical Center, Oklahoma City

Oklahoma Children's Memorial Hospital, Oklahoma City

Supervision of Students and Postdoctoral Fellows

Ph.D. Candidates

Mark J. Mamula	Ph.D.	1986	The role of the Ro/SSA ribonucleoprotein Microbiology as an immunogen: A study of the antigen binding properties
Myra O. Rosario	Ph.D.	1987	Studies on the immunology of the human Microbiology ribonucleoprotein autoantigen Ro/SSA
Kimberley K. Gaither	Ph.D.	1987	Anti-Ro/SSA in normal sera and its role Microbiology in the pathogenesis of neonatal lupus syndrome (R.A. Patnode Award, Sigma Xi Award)
Charles A. O'Brien	Ph.D.	1990	A subset of hY RNAs are associated with Microbiology erythrocyte Ro RNPs and the sequence of hY4 RNA (Sigma Xi Award)
Judith A. James	Ph.D.	1993	Sequential fine specificity of Sm and nRNP associated proteins
A. Darise Farris	Ph.D.	1995	Phylogenetic analysis of Ro ribonucleoprotein associated small RNAs
Kathy L. Moser	Ph.D.	1995	Genetic linkage analysis of chromosome 1 marker loci to systemic lupus erythematosus and anti-nuclear antibodies.
Melissa Arbuckle			Dissertation research underway

Summer Student Research Scholars

Michael D. Rader	1983	Sir Alexander Fleming Scholar
Andrea L. Sestak	1984	Sir Alexander Fleming Scholar
Ann J. Althizer	1985	Sir Alexander Fleming Scholar
E. Michele Southard	1986	Sir Alexander Fleming Scholar
Dedra Butler	1987	Sir Alexander Fleming Scholar
Julie Cleek	1988	OMRF Scholar

Judith Ann James	1988	Sir Alexander Fleming Scholar
Grace Yin Jenq	1989	Sir Alexander Fleming Scholar
Cy Anderson	1989	SURE Program Scholar
Holly Moreu	1990	Sir Alexander Fleming Scholar
David Stec	1990	SURE Program Scholar
Juliet von Egmond	1991	SURE Program Scholar
Kennith Layton	1991	Sir Alexander Fleming Scholar
W. Cody Holloway	1992	Sir Alexander Fleming Scholar
Tina Grover	1992	Presbyterian Harris Summer Fellow
Lee Warren	1992	Presbyterian Harris Summer Fellow
Melissa Arbuckle	1993	SURE Program Scholar
Monica S. Reid	1993	Sir Alexander Fleming Scholar
Rivka Galchen	1994	Sir Alexander Fleming Scholar
Robert Clay Musser	1994	Sir Alexander Fleming Scholar
Lydia D. Nightingale	1995	Sir Alexander Fleming Scholar
Micah T. McClain	1995	Sir Alexander Fleming Scholar
Audrey Brumback	1995, 1996	Summer Student
Rivka Galchen	1995	Summer Student
Connie Zhai	1995	Summer Student
Lydia Nightingale	1996, 1997, 1998	Summer Student
Teresa Hall	1996	Summer Student
Mark Goodman	1996	Summer Student
Jared Ning	1996, 1997	Summer Student
Jenifer Hahn	1996	Summer Student
Micah Mc Clain	1996	Summer Student
David Williams	1996	Summer Student
Alyssa Shilling	1997	Sir Alexander Fleming Scholar
Alan Lee	1997	Summer Student
Nelson Fong	1997, 1998	Summer Student
Anup Shetty	1998	Sir Alexander Flemming Scholar

Postdoctoral Fellows, Rheumatology Clinical Fellows and Research Scientists

Owen F. Fox, Ph.D.	1983-1987
Larry G. Willis, M.D.	1985-1986
Dan Axthelm, M.D.	1986-1987
Atsushi Fujisaku, M.D., Ph.D.	1986-1989
Kimberley K. Gaither, Ph.D.	1987-1988
W. Daryl Dickey, M.D., Ph.D.	1988-1991
R. Hal Scofield, M.D.	1988-1991
Bettina Mues, M.D., Ph.D.	1989-1990
Jose Troncoso, M.D.	1989-1991
Shu-Cai Huang, Ph.D.	1991-1993
Timothy Shaver, M.D.	1993-1994
Sangita Deveshwar, M.D.	1993-1995
Judith A. James, M.D., Ph.D.	1993-1995
Kenneth M. Kaufman, Ph.D.	1994-present
A. Darise Farris, Ph.D.	1995-1996

Kathy L. Moser, Ph.D.	1995-present
Iman Ali, M.D.	1997-present
Andrea Sestak, M.D., Ph.D.	1997-present

Faculty Support

Shaili Deveshwar, M.D.	1995-1996	Assistant Professor Department of Pediatrics University of Oklahoma
Kenneth M. Kaufman, Ph.D.	1994-present	Research Assistant Professor Department of Medicine University of Oklahoma
Judith A. James, M.D., Ph.D.	1995-present	Research Assistant Professor Department of Medicine University of Oklahoma
Barbara R. Neas, Ph.D.	1991-present	Assistant Professor Department of Biostatistics and Epidemiology University of Oklahoma
R. Hal Scofield, M.D.	1991-present	Assistant Professor Department of Medicine University of Oklahoma
	1991-present	Associate Member (1992-present), Assistant Member (1991-1992), Oklahoma Medical Research Foundation

Sabbatical and Visiting Scientists

Angela Horsfall, Ph.D.	1989-1990	Senior Scientist; Kennedy Institute for Rheumatology, London
Fanny Ebling, Ph.D.	1991	Associate Professor; Department of Medicine, University of California, Los Angeles
Jacob Karsh, M.D.	1992	Associate Professor; Department of Medicine, University of Ottawa, Ontario
Thomas Gordon, M.D.	1995-1996	Associate Professor; Department of Medicine, Flinders University Adelaide, Australia

Ramnath Misra, M.D.

1997

WHO Fellow
Additional Professor
Department of Clinical Immunology
Sanjoy Gandhi Postgraduate Institute
of the Medical Sciences
Lucknow, India

Research Support

Active Research Support

National Institutes of Health T32 AI07364-01 to 05

“Molecular Pathogenesis Training Program”

Principal Investigator: John Iandolo, Ph.D.

Training Personnel: J.B. Harley

2% time and effort

2/1/98 to 1/31/03: \$111,568 total direct costs

2/1/98 to 1/31/99: \$ 69,304 direct costs

National Institutes of Health T32 AR07580

“Immunological Training in Rheumatology and Dermatology”

Principal Investigator: M. Reichlin

Training Personnel: J.B. Harley

1% time and effort

7/1/94 to 6/30/99: \$ 328,900 total direct costs

7/1/98 to 6/30/99: \$ 65,520 direct costs

U.S. Department of Veterans Affairs, Merit Review Program, CC103

“Anti-Ro Autoantibody”

Principal Investigator: J.B. Harley

10% time and effort

10/1/94 - 9/30/99: \$465,500 total direct costs

10/1/98 - 9/30/99: \$ 93,100 direct costs

National Institutes of Health, Contract No. N01-AR-5-2221

“Lupus Multiplex Registry and Repository”

Principal Investigator: J.B. Harley

up to 20% time and effort

9/30/95 - 4/31/01: \$3,641,507 total costs

9/30/98 - 9/29/99: \$ 674,714 direct costs

National Institutes of Health K08 AR01981-01 to 05

“Genetic Analysis of Lupus Autoimmunity”

Principal Investigator: Judith A. James, M.D., Ph.D.

Mentor: J.B. Harley

9/30/96 - 8/31/01: \$378,722 total direct costs

9/30/98 - 8/31/99: \$ 71,460 direct costs

John B. Harley, M.D., Ph.D.

National Institute of Health R01 AI31584-04

"A Possible Infectious Etiology for Lupus"

Principal Investigator: J.B. Harley

4% time and effort

9/1/97 - 8/31/02: \$864,935 total direct costs

9/1/97 - 8/31/98: \$168,833 first year direct costs

National Institute of Health P50 AR45231-01

"Specialized Center of Research in Systemic Lupus Erythematosus"

Projects #2. "Multiplex and Simplex Pedigrees in the Genetics of Lupus"

Principal Investigator: Robert P. Kimberly, M.D.

Project #2 Investigator: J.B. Harley

20% time and effort

7/1/98 - 6/30/02: \$600,000 total direct costs

7/1/98 - 6/30/99: \$150,000 direct costs

Pending Research Support

National Institutes of Health RO1 AR42474-06 to 10

"Epstein-Barr Virus in Lupus"

Principal Investigator: J.B. Harley

2/1/99 - 1/31/04: \$1,006,331 total requested direct costs

2/1/99 - 1/31/00: \$185,796 requested direct costs

National Institutes of Health RO1 AR42460-06 to 10

"Genetic Association with Lupus in American Blacks"

Principal Investigator: J.B. Harley

4/1/99 - 3/31/04: \$2,026,214

4/1/99 - 3/31/00: \$351,272

Arthritis Foundation

"Epistasis with D1s229 in Lupus"

Principal Investigator: J.B. Harley

7/1/99 - 6/30/04: \$416,665 total requested direct costs

7/1/99 - 6/30/00: \$ 83,333 requested direct costs

U.S. Department of Veterans Affairs

"Research Enhancement Award"

Principal Investigator: J.B. Harley

1/1/99 - 6/30/99: \$ 40,000 direct costs

National Institutes of Health PO1 AI45756-01 to 05

Human Immunology Center of Excellence

"Pathogenic Mechanisms of Autoimmunity"

Principal Investigator: J. B. Harley

"Project 4, Potential Association of RA with Epstein-Barr Virus"

Project Investigator: J. B. Harley

8/1/99 - 7/31/04: \$640,731 total indirect costs

8/1/99 - 7/31/00: \$118,296 requested direct costs

Previous Research Support

U.S. Department of Veterans Affairs
"VAMC Genotyping and Sequencing Center"
Principal Investigator: J.B. Harley
7/20/98 - 9/30/98: 465,760

U.S. Department of Veterans Affairs
"Medical Research Service Equipment Request"
Principal Investigator: J.B. Harley
7/1/98 - 9/30/98: \$67,000

National Institutes of Health R01 AI24717-07 to 11
"Genetic Linkage in Lupus"
Principal Investigator: J.B. Harley
9/30/93 to 8/31/98: \$537,746

National Institutes of Health R01 AR42474
RFA AR-93-005 "Research on Causal Mechanisms in Systemic Lupus Erythematosus"
"Peptides Induce Lupus Autoimmunity"
Principal Investigator: J.B. Harley
9/30/93 to 8/31/98: \$547,843

National Institutes of Health
"Genetic Linkage in Lupus Research Supplement for a Minority Postdoctoral Trainee"
Principal Investigator: J.B. Harley
10/1/95 - 8/31/98: \$117,630

National Institute of Health T32 GM08237-08 to 10
"Septic Shock And Tissue Injury"
Principal Investigator: Gary T. Kinasewitz, M.D.
Training Personnel: J.B. Harley
7/1/96 to 6/30/97 \$69,100
7/1/97 to 6/30/98 \$72,000 total direct costs

National Institutes of Health R01 AR42460
RFA AR-93-006 "Systemic Lupus in Women and Minorities"
"A Genetic Association with Lupus in American Blacks"
Principal Investigator: J.B. Harley
9/30/93 to 5/31/98: \$504,504 total direct costs

National Institutes of Health T32 AI07364-01 to 05
"Molecular Pathogenesis Training Program"
Principal Investigator: Joseph J. Ferretti, Ph.D.
Training Personnel: J.B. Harley
2/1/93 to 1/31/98: \$226,994

National Institutes of Health K11 AR01844

John B. Harley, M.D., Ph.D.

Physician Scientist Award

“Origin of Anti-Ro/SSA Antibody”

Principal Investigator: Robert Hal Scofield, M.D.

Sponsor: J.B. Harley

3/1/92 to 2/28/97: \$335,000

National Institutes of Health R01 AR42474-S1

Research Supplement for Minority Investigators

“Peptides Induce Lupus Autoimmunity”

Principal Investigator: J.B. Harley

Co-Principal Investigator: J.A. James

2/1/95 to 1/31/97: \$134,610

National Institutes of Health F31 GM14841

“MARC Predoctoral Fellowship”

Judith A. James, Fellowship Recipient

John B. Harley M.D., Ph.D., Sponsor

10/1/91 to 9/30/96: \$81,800

National Institutes of Health R01 AI31584

“Y RNA Sequences and Ro RNP Function”

Principal Investigator: J.B. Harley

7/1/91 to 6/30/96: \$155,655

U.S. Department of Veterans Affairs, Merit Review Program CC103

“Human Anti-Ro/SSA Producing Xenografts”

Principal Investigator: J.B. Harley

10/1/91 to 9/30/94: \$173,794

National Institutes of Health, Program Project, P01 AI21568-06 to 08

“Autoantibodies in SLE and Polymyositis”

Program Director: Morris Reichlin, M.D.

9/1/90 to 6/30/94: \$1,253,561 total approved direct costs

Core C

“Experimental Design and Statistical Analysis”

Principal Investigator: J.B. Harley

9/1/90 to 7/31/94: \$39,599

Project 3

“Autoantigenicity of Sm and nRNP”

Principal Investigator: J.B. Harley

9/1/90 to 7/31/94: \$221,829

Presbyterian Health Foundation

MD/PhD Program

Principal Investigator: J.B. Harley

11/1/92 to 10/30/93: \$104,000 approved direct costs

National Institute of Health, R01 AI24717-04 to 06

"HLA-DQ Gene Complementation in Lupus"

Principal Investigator: J.B. Harley

4/1/90 to 8/31/93: \$241,338

National Institutes of Health, T32 GM08237

"Septic Shock and Tissue Injury"

Principal Investigator: Lerner Hinshaw, Ph.D.

Supporting Investigator: J.B. Harley

7/1/88 to 6/30/93: \$190,242

National Institutes of Health, R01 AR39577-01 to 03

"Antiidiotypes in Congenital Heart Block and Neonatal Lupus"

Principal Investigator: J.B. Harley

2/1/89 to 3/31/93: \$239,000

Veterans Administration Clinical Investigator Award (CC108)

"HLA Gene Complementation in Systemic Lupus"

Principal Investigator: J.B. Harley

7/1/87 to 3/31/93: \$585,000

Arthritis Foundation, Oklahoma City Chapter

"Mouse and Rabbit Y RNA Sequences"

Principal Investigator: J.B. Harley

11/1/90 to 10/31/91: \$9,260

OCAST Equipment Contract

"Autoimmune Responses in Polymyositis"

Principal Investigator: M. Reichlin

Co-Investigator: J.B. Harley

9/1/89 to 8/30/90: \$26,177

March of Dimes Birth Defects Foundation (1-1109)

"Immunopathogenesis of Congenital Heart Block"

Principal Investigator: J.B. Harley

7/1/88 to 6/30/90: \$60,000

National Institutes of Health, R01 AI24717

"HLA Gene Complementation in Primary Sjögren's and Lupus"

Principal Investigator: J.B. Harley

Co-Principal Investigator: M. Barton Frank, Ph.D.

4/1/87 to 6/30/90: \$244,537

Arthritis Foundation - Arthritis Investigator Award

"Autoimmune Epitopes of Ro/SSA I."

Principal Investigator: J.B. Harley

7/1/85 to 6/30/88: \$108,000

College of Medicine Alumni Research Fund

"Autoimmune Epitopes of Ro/SSA II."

Principal Investigator: J.B. Harley
7/01/87 to 1/31/89: \$18,296

National Institutes of Health AM34159
"Immunology of the Autoantigen Ro/SSA and La/SSB"
Principal Investigator: J.B. Harley
4/1/85 to 3/31/88: \$ 150,000

National Institutes of Health, AM 34159
"Immunology of the Autoantigens Ro/SSA and La/SSB"
Principal Investigator: J.B. Harley
4/1/85 to 3/30/88: \$107,500

NATO Scientific Affairs Division, RG 86/0564
Collaborative Research Grant Program RG.86/0564
"Autoantibodies in Complete Congenital Heart Block"
Project Coordinator: J.B. Harley
3/86 to 12/87: \$5,000

Veterans Administration Medical Research Service,
CC 103 (Merit Review)
"RNA-Protein in Autoimmune Diseases"
Principal Investigator: J.B. Harley
1/1/86 to 12/31/87: \$95,400

March of Dimes Birth Defects Foundation,
Basil O'Connor Starter Research Grant
"Anti-Ro/SSA and Congenital Complete Heart Block"
Principal Investigator: J.B. Harley
9/1/85 to 8/31/87: \$50,000

National Institutes of Health, R01 DE 06740
"Human Antibody Responsiveness and Dental Caries"
Principal Investigator: Martin Levine, Ph.D.
Co-Investigator: J.B. Harley
7/1/84 to 6/30/87: \$207,445

Veterans Administration Medical Research Service, CC 103 (RAG)
"Autoantigen Recovery in Autoimmune Disease"
Principal Investigator: J.B. Harley
1/1/85 to 12/31/85: \$25,000

Oklahoma Chapter Arthritis Foundation
"Monoclonal Antibodies Against Ro/SSA"
Principal Investigator: J.B. Harley
7/1/84 to 6/30/85: \$5,500

Oklahoma Lupus Association, Inc.
"Anti-Ro/SSA Autoantibodies in Normal Individuals"

Principal Investigator: J.B. Harley
6/1/84 to 5/31/85: \$3,500

Oklahoma Chapter, Arthritis Foundation
"Does Epstein-Barr Virus Cause Rheumatoid Arthritis?"
Principal Investigator: J.B. Harley
7/1/82 to 6/30/83: \$3,500

Oklahoma Chapter, Arthritis Foundation
"Immunohistochemical Identification of the Isoenzymes of G6PD"
Principal Investigator: J.B. Harley
7/1/82 to 6/30/83: \$8,500

National Institutes of Health, AI 05186
"Phagocytosis and Lipid Acyl Chain Composition in *Escherichia Coli*"
Principal Investigator: J.B. Harley
7/1/75 to 7/1/77: \$26,000

Patents

U.S. Patent No. 4,784,942 entitled "Monoclonal antibodies against autoimmune RNA proteins,"
issued November 15, 1988. Inventor, J.B. Harley

U.S. Patent No. 5,264,351 entitled "Monoclonal antibodies against autoimmune RNA proteins,"
issued November 23, 1993. Inventor, J.B. Harley

U.S. Patent No. 5,637,454 entitled "Assays and treatments for autoimmune diseases." Issued June 10,
1997. Inventor, J.B. Harley [OMRF 114]

U.S. Patent No. 5,719,064 entitled "Peptide diagnostics and therapeutics for spondyloarthropathies".
Issued February 17, 1998. Inventors R.H. Scofield and J.B. Harley.

Continuation in Part U.S. Patent applied for "Assays and treatments for autoimmune diseases."
Provisional U.S. Serial Number: 08/335,198, Unofficial Filing Date April 13, 1992. Inventor, J.B.
Harley [OMRF 116 CIP (1)], Notice of allowance issued in October 24, 1996 for claims 1-8, 10, 64-
65.

Continuation in Part U.S. Patent applied for "Methods and reagents for diagnosis of autoantibodies."
Provisional U.S. Serial Number: 07/867,819, Unofficial Filing Date June 22, 1992. Inventor, J.B.
Harley [OMRF 114 CIP (2)]

U.S. Patent applied for "Peptide diagnostics and therapeutics for spondyloarthropathies." Provisional
U.S. Serial Number: 07/944,143, Unofficial Filing Date August 31, 1992. Inventors, R. Hal Scofield
and J.B. Harley [OMRF 138] Patent issued in U.S.A.

Continuation in Part U.S. Patent applied for "Peptide and induction of autoimmunity and clinical
symptomology." Provisional U.S. Serial Number 08/160,604 Unofficial Filing Date, November 30,
1993. Inventors, J.B. Harley, J.A. James and R.H. Scofield [OMRF 114 CIP (3)] "Applications
pending in Europe (93910594.6), Japan (5-518573), Australia (41028193) and Canada (2,117,904).

U.S. Patent applied for "Diagnostics and therapy of Epstein-Barr virus in autoimmune disorders".
Provisional U.S. Serial Number pending. Inventors, J.B. Harley and J.A. James [OMRF 161]
Unofficial filing date January 13, 1997.

Bibliography

GenBank Sequence Database Submissions

1. O'Brien, C.A. and J.B. Harley. Human hY4 Ro RNA. Feb. 27, 1991. Accession Number: X57566; Locus: HSHY4RO
2. O'Brien, C.A. and J.B. Harley. H. Sapien (clones B7, B8 and F2) hY4 Ro RNA pseudogenes. Dec. 14, 1992. Accession Numbers: M77130-M77132; Locus: HUMHY4ROA, HUMHY4ROB and HUMHY4ROC.
3. Farris, A.D. and J.B. Harley. Iguana small cytoplasmic RNA (Y3 and Y4). Jan. 11, 1994, Accession Numbers: L27530-L27537; Loci: IGUY3A, IGUY3B, IGUY3C, IGUY3D, IGUY4A, IGUY4B, IGUY4C, IGUY4D. Corrections submitted August 11, 1994.

Publications

1. Harley, J.B. Oxygen toxicity and lipid oxidation in *Escherichia coli*. Dissertation, University of Pennsylvania, 1976.
2. Harley, J.B., Grinspan, S. and Root, R.K. Paraquat suicide in a young woman: Results of therapy directed against the superoxide radical. *Yale J. Biol. Med.* 50:481-488, 1977.
3. Harley, J.B., Santangelo, G.M., Rasmussen, H. and Goldfine, H. Dependence of *Escherichia coli* hyperbaric oxygen toxicity on the lipid acyl chain composition. *J. Bacteriol.* 134:808-820, 1978.
4. Goldfine, H., Harley, J.B. and Wyke, J.A. Effects of inhibitors of lipid synthesis on the replication of Rous sarcoma virus. A specific effect of cerulenin on the processing of major non-glycosylated viral structural proteins. *Biochim. Biophys. Acta* 512:229-240, 1978.
5. Harley, J.B. and Goldfine, H. Effects of lipid synthesis on transformation in chicken embryo fibroblasts infected with Rous sarcoma virus. *Exp. Cell Res.* 118:47-54, 1979.
6. Gealy, W.J., Dwyer, J.M. and Harley, J.B. Allelic exclusion of glucose- 6-phosphate dehydrogenase in platelets and T lymphocytes from a Wiskott-Aldrich syndrome carrier. *Lancet* 1:63-65, 1980.
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9. Harley, J.B. Clinical manifestations of patients with hypereosinophilic syndrome. In The Idiopathic Hypereosinophilic Syndrome: Clinical, Pathophysiologic and Therapeutic Considerations (A.S. Fauci, moderator), pp. 82-84. Ann. Intern. Med. 97:78-92, 1982.
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14. Harley, J.B., Fauci, A.S. and Gralnick, H.R. Noncardiovascular findings associated with heart disease in the idiopathic hypereosinophilic syndrome. Am. J. Cardiol. 52:321-324, 1983.
15. Harley, J.B. and Fauci, A.S. Cyclosporine modulates the human in vitro T-dependent antigen-induced synthesis of specific antibody. Transplant. Proc. 15 (Suppl. 1):2315-2320, 1983.
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John B. Harley, M.D., Ph.D.

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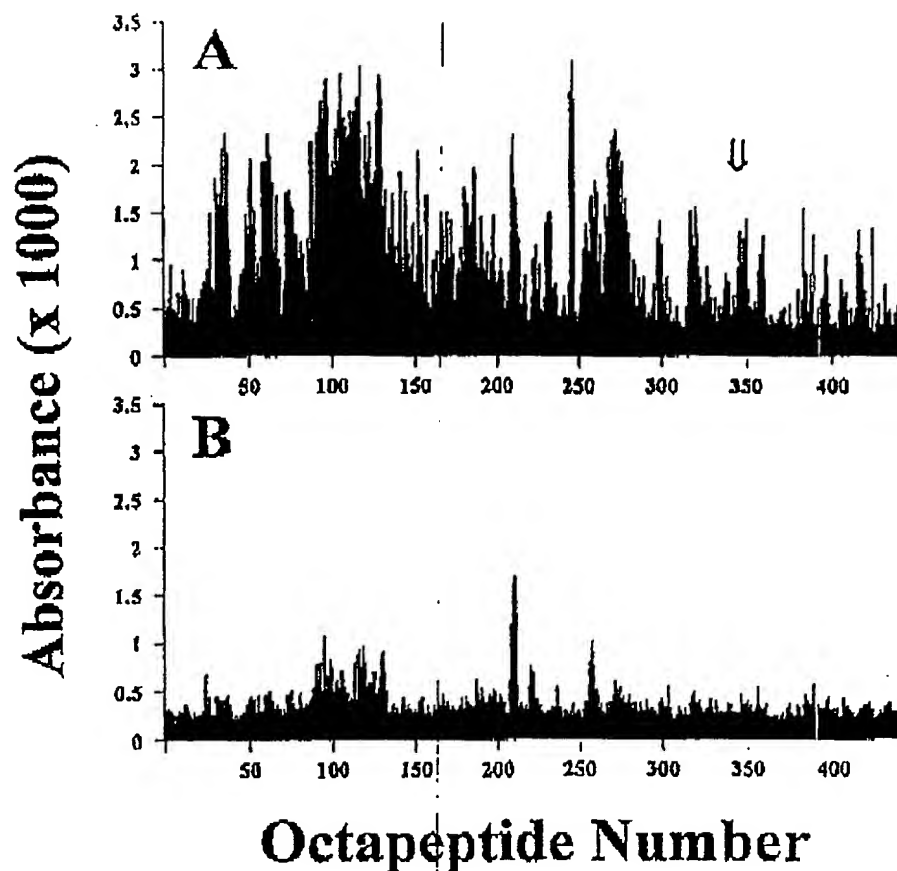


Figure 1. Binding of a rheumatoid factor positive RA patient serum (A) and a sex, race and age matched negative control (B) to the 443 unique overlapping octapeptides of EBNA-1. The RA patient mounts a very diversified response against EBNA-1. The normal control, on the other hand, has a much more limited response. Note the apparently identical responses binding peptides near peptide number 210 and 260 in both the normal and the RA patient. Meanwhile, the RA patient has anti-EBNA-1 antibodies against a number of peptides not bound by the normal. Among these are octapeptides numbered 344 to 347 (↓) which contain RLPFG which is crossreactive with collagen (Cook, et al. J. Autoimmun. 11:205-211 (1998)).

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Table 2. Various immunization schedules for peptide induced lupus autoimmunity. Sch. A and Sch. B are presented in the *J. Exp. Med.* paper listed above and the Sch. B* is presented in the *J. Invest. Med.* paper also referenced above.

	<u>INJECTIONS (Days from 1st)</u>								
	1 st	2 nd	3 rd	4 th	5 th	6 th	7 th	8 th	9 th
Schedule A	0	26	53	99	152				
Schedule B	0	8	22	67	151	275	317		
Schedule B*	0	8	57	99	155	248	276	291	312
Schedule C	0								

The first injection is given on Day 0 for all animals.

EXHIBIT 3

